

## **TITLE OF THE INVENTION**

**[0001]**      Rapidly Changing Dichroic Beamsplitter

## **CROSS REFERENCE TO RELATED APPLICATIONS**

**[0002]**      This application claims priority from United States Provisional Patent Application no. 60/249,626, filed November 17, 2000, and is a continuation International Patent Application PCT/US01/42957. The entire contents of both of the aforementioned patent applications are incorporated herein by reference.

## **BACKGROUND OF THE INVENTION**

**[0003]**      This invention relates to epi-fluorescent microscopy, and more particularly to the rapid selection of filters for measuring fluorescence at different wavelengths or Stokes shifts.

**[0004]**      Current fluorescent microscope designs employ an incident light or epi-fluorescent design where a dichroic beam splitter (or chromatic mirror) mounted in a filter cube at a 45 degree angle to the excitation light path, is used to reflect shorter excitation wavelengths of light onto the specimen while passing longer emission wavelengths to the eyepieces or camera (Figure 2).

**[0005]**      Many fluorescence applications require two or more fluorescent labels to be present in the specimen. Each label has its own excitation and emission spectra, and thus requires different excitation and emission filters, as well as a different dichroic beamsplitter.

[0006] To date two approaches to using multiple fluorophores have been employed:

- 1) Use dichroic beamsplitters that have multiple cutoff wavelengths. Thus a single dichroic can be used with multiple fluorophores. However because of bandwidth restrictions, total light throughput is reduced, thus creating longer exposure times when working with a camera. Longer exposure times translate into longer acquisition duty cycles which is problematic in paradigms requiring repetitive high-speed data acquisition (screening applications and applications using living cells).
- 2) Motorized filter cube changers. Several commercially available microscopes employ motorized filter cube changes. These allow the use of single dichroics for each fluorophore. However switching time is slow (1-2 seconds) which creates problems when using multiple fluorophores in paradigms requiring repetitive high-speed data acquisition (screening applications and applications using living cells).

## SUMMARY OF INVENTION

[0007] Disclosed below is a device for rapidly changing dichroic beamsplitters in epi-fluorescent microscopes. The device is a high speed wheel in which dichroic beamsplitters are mounted. The high speed dichroic changer is mounted in an epi-fluorescent microscope, and the changer is under computer control. Computer software can command the changer to rotate different dichroic beamsplitters into the epifluorescent lightpath so that the appropriate dichroic is in position when a particular fluorophore is imaged. The present invention provides a microscope system in which the device is commanded by the software to change dichroic beamsplitters (Figure 1).

## BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Figure 1 is a system schematic showing some key components of an analysis microscope constructed according to the present invention.

[0009] Figure 2 is a schematic drawing showing epi-fluorescent light paths occurring in the system of Figure 1.

[0010] Figure 3 is a top view showing the arrangement of a high speed dichroic beamsplitter wheel constructed according to the present invention.

[0011] Figure 4 is a schematic drawing showing control of a microscope according to one aspect of the invention.

## DETAILED DESCRIPTION

[0012] Figure 1 is a system schematic showing some key components of an analysis microscope 11 constructed according to one aspect of the invention. The microscope includes a light source 12, a dichroic beamsplitter wheel 13, and an objective 14 which is aligned with a specimen 15. Light from the light source 12 is reflected at the dichroic wheel 13, and passes through the objective 14. Light reflected or fluorescing from the specimen 15 again passes through the objective 14, and that light which passes through the dichroic wheel 13 is received by the camera 16. Still referring to Figure 1, the dichroic wheel 13 is rotationally positioned by a stepper motor 17, which is controlled by motor controller 18. The motor controller 18 is responsive to a computer 19, and the computer 19 also receives images from the camera 16.

[0013] Figure 2 is a schematic drawing showing epi-fluorescent light paths. Light from the light source 12 is reflected by the dichroic beamsplitter wheel 13 which is at a reflection angle which determines the lightpath. In the exemplary embodiment, a 45

degree angle is used as the reflection angle, although since standard dichroic cubes with the 45 degree angle are not used, it is likely that other reflection angles can be used. The return light, if it is able to pass the dichroic wheel 13, is received by the camera. This sequence generally corresponds to fluorescent microscope designs which employ a dichroic beam splitter or a chromatic mirror mounted in a filter cube at a 45 degree angle to the excitation light path. Significantly the filter cube is not required.

**[0014]** Figure 3 is a top view showing the arrangement of a high speed dichroic beamsplitter wheel 13 constructed according to one embodiment of the invention. The wheel 13 includes a support plate 52, on which a plurality of dichroic beamsplitter lenses 61-65 are arranged about a center axis 66 of the support plate 52. Each of the lenses 61-65 have distinct filtering properties. In many cases, only two lenses are required, so it is possible to use plano lenses in the remaining three spots, or to leave the remaining three spots empty.

**[0015]** The solution described here is to mount round 50 mm dichroic beamsplitters in a high-speed filter wheel (Figure 3). Any size or shape dichroic beamsplitter that matched the optical path of the microscope or instrument would be acceptable.

**[0016]** The wheel is capable of switching between adjacent dichroics in 50 msec. The wheel in the current implementation is driven by a DC stepper motor and is under computer control. Switching time is a function of wheel mass and motor speed. Any motor-wheel combination that allowed faster switching times would be acceptable. It is also conceivable that a galvanometer could drive a wheel at much higher speeds, or that a galvanometer could also move dichroic beamsplitters mounted on a spindle.

**[0017]** The wheel is mounted at a 45 degree angle inside the microscope such that when a given dichroic is selected the opening holding that dichroic is rotated into the epi-fluorescent light path, and the selected dichroic beamsplitter is at 45 degree angle to the

excitation light (figure 1, figure 4).

**[0018]** This design this allows optimized throughput for each fluorophore while at the same time being able to switch the dichroics rapidly. Optimized throughput is advantageous as it allows for shorter camera exposure times, which decreases duty cycle as well as helps reduce photobleaching. Being able to rapidly switch the dichroic beamsplitter reduces the interval between image acquisitions, thus reducing duty cycle.

**[0019]** Figure 4 is a schematic drawing showing control for a microscope using a fast dichroic beamsplitter changer according to one aspect of the invention. The figure depicts a loop 80, which represents one complete duty cycle with respect to a predetermined wavelength. Application software sends a move command 82 to a dichroic beamsplitter wheel controller such as controller 18 in Figure 1. The controller firmware then interprets 83 the move command and rotates 83 the wheel (13, Figure 1) to a correct position. The controller 18 then signals 84 application software that wheel 13 has finished moving. In response, the computer (19, Figure 1) signals 85 the camera (16, Figure 1) to acquire 86 an image. The image is then acquired 87.

**[0020]** This sequence repeated by executing a loop 80 for every wavelength required.